UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/779,560	02/09/2001	Marianne Harboe	58982.000002	6162

7590 06/18/2010

Stanislaus Aksman
Hunton & Williams
Suite 1200
1900 K Street, N.W.
Washington, DC 20006

EXAMINER	
STEADMAN, DAVID J	

ART UNIT	PAPER NUMBER
1656	

MAIL DATE	DELIVERY MODE
06/18/2010	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	09/779,560	HARBOE, MARIANNE	
	Examiner	Art Unit	
	David J. Steadman	1656	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *23 March 2010*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *44-78* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *44-78* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____ .

DETAILED ACTION

Status of the Application

[1] Claims 44-78 are pending in the application.

[2] Applicant's amendment to the claims, filed on 3/23/10, is acknowledged. This listing of the claims replaces all prior versions and listings of the claims.

[3] Applicant's remarks filed on 3/23/10 in response to the non-final Office action mailed on 9/24/09, have been fully considered and are deemed to be persuasive to overcome at least one of the rejections and/or objections previously applied. Rejections and/or objections not reiterated from previous Office actions are hereby withdrawn.

[4] The text of those sections of Title 35 U.S. Code not included in the instant action can be found in a prior Office action.

Claim Objection

[5] The objections to claims 44, 48, 51, 60, and 77 are withdrawn in view of the instant amendment to the claims.

Claim Rejections - 35 USC § 112, First Paragraph

[6] The written description rejection of claims 57, 59, 73, and 75 under 35 U.S.C. 112, first paragraph, is withdrawn in view of the instant claim amendment to delete the term "derived", thus limiting the gene to encoding *Camelus dromedarius* chymosin (claims 57 and 73) or limiting the gene to encoding bovine chymosin (claims 59 and 75).

[7] The written description rejection of claims 44-56, 58, 60-72, 74, and 76-77 under 35 U.S.C. 112, first paragraph, is maintained for the reasons of record. The rejection was fully explained in a prior Office action. See [9] beginning at p. 3 of the Office action mailed on 9/24/09. Newly added claim 78 is included in the rejection as reciting, "a gene encoding chymosin from a...*Camelidae* species". *Thus claims 44-56, 58, 60-72, 74, and 76-78 are rejected herein.*

RESPONSE TO ARGUMENT: Beginning at p. 7 of the instant remarks, applicant argues the claims are drawn to methods and not chymosin *per se* and the methods can be practiced irrespective of the recited chymosin.

Applicant's argument is not found persuasive. The examiner maintains the position that the specification and prior art adequately describe genes encoding bovine or *Camelidae dromedarius* chymosin, the specification and prior art fail to adequately describe genes encoding all *Camelidae* chymosins. Addressing an argument regarding the distinction between the written description of a compound and the written description of a method of using that compound, the Court in *University of Rochester v. G.D. Searle & Co.* 69 USPQ2d 1886 (CAFC 2004) stated, "[r]egardless whether a compound is claimed per se or a method is claimed that entails the use of the compound, the inventor cannot lay claim to that subject matter unless he can provide a description of the compound sufficient to distinguish infringing compounds from non-infringing compounds, or infringing methods from non-infringing methods. Thus, contrary to applicant's position, the genus of recited genes encoding chymosin from

a…*Camelidae* species" must be adequately described to satisfy the written description requirement of 35 U.S.C. 112, first paragraph.

Applicant argues the prior art provides a correlation between the structures of genes encoding all chymosins from any *Camelidae* species and chymosin enzymatic activity. According to applicant, the prior art would consider a *Camelidae dromedarius* chymosin as being representative of chymosin from all other *Camelidae* species because: there is a close structural relationship among chymosins from the six *Camelidae* species; three "areas of special interest" in bovine chymosin are also present in *Camelidae* species chymosin; and the amino acid sequences of cytochrome b and hemoglobin alpha and beta chains are closely related among certain *Camelidae* species.

Applicant's argument is not found persuasive. Absent evidence that the nucleic acid and/or amino acid sequences of chymosin from the six *Camelidae* species other than *Camelidae dromedarius* were known at the time of the invention, applicant's argument regarding an alleged close structural relationship among chymosins from the six *Camelidae* species and that three "areas of special interest" in bovine chymosin are also present in *Camelidae* species chymosin amounts to hindsight analysis and reasoning, relying on evidence that was available only *after* the time of the invention. Because applicant's position relies on post-filing evidence that was not available at the time of the invention, it appears that the specification and the state of the art *at the time of the invention* fail to support a disclosed or art-recognized correlation between the structure of a gene encoding *Camelidae dromedarius* chymosin and genes encoding

chymosin from any other *Camelidae* species. Moreover, that the sequences of cytochrome b and hemoglobin alpha and beta chains are also closely related among certain *Camelidae* species is merely coincidental and provides no indication as to the structures, *i.e.*, amino acid sequences, of chymosin from other *Camelidae* species, whose sequences appear to be entirely unrelated to the amino acid sequences of cytochrome b and hemoglobin alpha and beta chains.

[8] Claim 77 is newly rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new matter rejection and is necessitated by the instant claim amendment.

MPEP § 2163.II.A.3.(b) states, "when filing an amendment an applicant should show support in the original disclosure for new or amended claims" and "[i]f the originally filed disclosure does not provide support for each claim limitation, or if an element which applicant describes as essential or critical is not claimed, a new or amended claim must be rejected under 35 U.S.C. 112, para. 1, as lacking adequate written description". According to MPEP § 2163.I.B, "While there is no *in haec verba* requirement, newly added claim limitations must be supported in the specification through express, implicit, or inherent disclosure" and "The fundamental factual inquiry is whether the specification conveys with reasonable clarity to those skilled in the art that,

as of the filing date sought, applicant was in possession of the invention as now claimed. See, e.g., *Vas-Cath, Inc.*, 935 F.2d at 1563-64, 19 USPQ2d at 1117".

Claim 77 is amended to recite, "The method of claim 60, wherein the glucoamylase is derived from an *Aspergillus* species". The specification's only description of the meaning of the term "derived from" with respect to a polypeptide is directed to an aspartic protease, not glucoamylase. According to the specification, the meaning of "derived from" with respect to an aspartic protease is "the addition or deletion of one or more amino acids or substituting one or more amino acids" (p. 8, lines 32-35). As such, in view of the disclosure's description of "derived from" with respect to an aspartic protease, claim 77 is interpreted as meaning the recited glucoamylase has "the addition or deletion of one or more amino acids or substituting one or more amino acids" relative to an *Aspergillus* species glucoamylase. However, this genus of glucoamylase polypeptides does not appear to be adequately supported by the original application. Applicant is invited to show support for claim 77.

[9] Claim 77 is newly rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a written description rejection and is necessitated by the instant claim amendment.

According to MPEP 2163.II.A.1, in evaluating a claimed invention for adequate written description, the examiner should determine what the claim as a whole covers. "Claim construction is an essential part of the examination process. Each claim must be separately analyzed and given its broadest reasonable interpretation in light of and consistent with the written description. See, e.g., *In re Morris*, 127 F.3d 1048, 1053-54, 44 USPQ2d 1023, 1027 (Fed. Cir. 1997)."

Claim 77 limits the genus of polypeptides having glucoamylase activity of the method of claim 60 to being "derived from an *Aspergillus* species". As noted above, the specification's only description of the meaning of the term "derived from" with respect to a polypeptide is directed to an aspartic protease, not glucoamylase. According to the specification, the meaning of "derived from" with respect to an aspartic protease is "the addition or deletion of one or more amino acids or substituting one or more amino acids" (p. 8, lines 32-35). As such, in view of the disclosure's description of "derived from" with respect to an aspartic protease, claim 77 is interpreted as meaning the recited glucoamylase has "the addition or deletion of one or more amino acids or substituting one or more amino acids" relative to an *Aspergillus* species glucoamylase, which encompasses a structurally unlimited glucoamylase polypeptide, including naturally-occurring glucoamylases as well as mutant and variant forms thereof, where 50% of glucoamylase activity is inactivated at a pH between 1.0 and 1.7.

For claims drawn to a genus, MPEP § 2163 states the written description requirement for a genus may be satisfied through sufficient description of a representative number of species by actual reduction to practice, reduction to drawings,

or by disclosure of relevant, identifying characteristics, i.e., structure or other physical and/or chemical properties, by functional characteristics coupled with a known or disclosed correlation between function and structure, or by a combination of such identifying characteristics, sufficient to show the applicant was in possession of the claimed genus. MPEP § 2163 states that a representative number of species means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus. In this case, the specification discloses only a single representative species of the recited genus of glucoamylase polypeptides, *i.e.*, *Aspergillus niger* var. *awamori* glucoamylase, where 50% of glucoamylase activity is inactivated at a pH between 1.0 and 1.7. Other than this disclosed species, the specification fails to disclose other representative species of the genus of recited glucoamylase polypeptides where 50% of glucoamylase activity is inactivated at a pH between 1.0 and 1.7. In this case, the genus of glucoamylase polypeptides encompasses widely variant species, including, but not limited to naturally occurring glucoamylases from any species and any mutants and variants thereof. The disclosure of the single representative species as noted above fails to reflect the variation among the members of the genus.

Therefore, given the lack of description of a representative number of compounds, the specification fails to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize that appellant was in possession of the claimed invention.

[10] The scope of enablement rejection of claim(s) 44-77 under 35 U.S.C. 112, first paragraph, is withdrawn. The rejection as it applied to claims 57, 59, 73, and 75 is withdrawn in view of the instant claim amendment to delete the term "derived", thus limiting the gene to encoding *Camelus dromedarius* chymosin (claims 57 and 73) or limiting the gene to encoding bovine chymosin (claims 59 and 75). The rejection as it applied to claims 44-56, 58, 60-72, 74, and 76-77 is withdrawn in view of the instant claim amendment to delete the term "that is derived" in claims 44 and 60, thus limiting the gene to encoding bovine or *Camelus* species chymosin. As noted in the prior Office action, applicant presents evidence that an mRNA encoding a wild-type *Camelus dromedarius* chymosin was known in the art at the time of the invention as evidenced by EMBL accession number AJ131677 and further provides evidence that an *Aspergillus niger* transformed with an expression vector encoding wild-type *Camelus dromedarius* chymosin was known at the time of the invention as evidenced by US Patent 7,270,989 (*e.g.,* paragraph bridging pp. 9-10 of the Office action mailed on 9/24/09). Methods for isolating naturally-occurring nucleic acids encoding polypeptides with known and routinely assayed enzymatic activities were known in the prior art at the time of the invention and routinely practiced. As such, it is the examiner's position that it would have required no more than routine experimentation to isolate genes encoding chymosin from other *Camelus* species.

[11] Claim 77 is newly rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method using a medium having *Aspergillus niger* glucoamylase activity and subjecting the medium to a pH of 1.0 to 1.7 to inactivate at least 50% of the glucoamylase activity, does not reasonably provide enablement for methods as broadly encompassed by the claims. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

"The test of enablement is not whether any experimentation is necessary, but whether, if experimentation is necessary, it is undue." *In re Angstadt*, 537 F.2d 498, 504, 190 USPQ 214, 219 (CCPA 1976). Factors to be considered in determining whether undue experimentation is required are summarized in *In re Wands* (858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988)) as follows: (A) The breadth of the claims; (B) The nature of the invention; (C) The state of the prior art; (D) The level of one of ordinary skill; (E) The level of predictability in the art; (F) The amount of direction provided by the inventor; (G) The existence of working examples; and (H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. See MPEP § 2164.01(a). The Factors most relevant to the instant rejection are addressed in detail below.

The breadth of the claims: As noted above, claim 77 limits the scope of polypeptides having glucoamylase activity of the method of claim 60 to being "derived from an *Aspergillus* species". As further noted above, the specification's only description of the meaning of the term "derived from" with respect to a polypeptide is directed to an

aspartic protease, not glucoamylase. According to the specification, the meaning of "derived from" with respect to an aspartic protease is "the addition or deletion of one or more amino acids or substituting one or more amino acids" (p. 8, lines 32-35). As such, in view of the disclosure's description of "derived from" with respect to an aspartic protease, claim 77 is interpreted as meaning the recited glucoamylase has "the addition or deletion of one or more amino acids or substituting one or more amino acids" relative to an *Aspergillus* species glucoamylase, which encompasses a structurally unlimited glucoamylase polypeptide, including naturally-occurring glucoamylases as well as mutant and variant forms thereof, where 50% of glucoamylase activity is inactivated at a pH between 1.0 and 1.7.

The nature of the invention: As acknowledged by the instant specification, in the production of recombinant chymosin, additional undesired activities are also present in the culture medium, including situations where "the desired product is produced as a fusion protein…and a fusion partner...having...an undesired enzymatic side activity" (specification at p. 1), wherein the specification discloses the specific embodiment of bovine chymosin fused to *Aspergillus niger* var. *awamori* glucoamylase fusion protein as an example thereof (specification at p. 9, bottom). The invention involves reducing the pH of a culture medium comprising chymosin and glucoamylase to remove unwanted glucoamylase activity, while maintaining chymosin activity.

The state of the prior art; The level of one of ordinary skill; The level of predictability in the art: Methods for reducing unwanted side activities in a microbial culture medium by lowering pH were well-known at the time of the invention. See, *e.g.,*

Lausten (US Patent 6,080,564, June 2000; cited in the PTO-892 filed on 4/9/02). Also, methods of recombinant production of bovine chymosin using a microbial expression host were well-known at the time of the invention. See, *e.g.,* Lawlis, Jr. et al. (US Patent 5,801,034, particularly column 2, lines 58-64; cited in the PTO-892 filed on 12/11/06) and Ward et al. (*Biotechnol* 8:435-440, abstract; cited in the IDS filed 16 April 2001).

Regarding the mutant and variant genes as encompassed by the claims, it is noted that the nucleotide sequence of an encoding nucleic acid determines the corresponding encoded protein's structural and functional properties, including its ability to maintain activity under a given set of conditions, *e.g.,* pH. Predictability of which changes can be tolerated in an encoded protein's amino acid sequence and obtain the desired activity requires a knowledge of and guidance with regard to which amino acids in the protein's sequence, if any, are tolerant of modification and which are conserved (i.e., expectedly intolerant to modification), and detailed knowledge of the ways in which the proteins' structure relates to its function. The positions within an encoding nucleic acid's sequence where modifications can be made with a reasonable expectation of success in obtaining an encoded polypeptide having the desired activity/utility are limited in any protein and the result of such modifications is highly unpredictable. In addition, one skilled in the art would expect any tolerance to modification for a given protein to diminish with each further and additional modification, e.g. multiple substitutions. As noted in prior Office actions, the state of the art provides evidence for the high level of unpredictability in altering a polynucleotide sequence with an expectation that the encoded polypeptide will maintain the desired activity/utility.

The amount of direction provided by the inventor; The existence of working examples: The specification discloses an analysis of *Aspergillus niger* glucoamylase in an *Aspergillus niger* var. *awamori* culture medium at pH 1.6, 1.7, 1.8, and 5.6 after 21 hours. See Tables 2.1 and 2.2 at pp. 12-13 of the instant specification. The disclosed experimental evidence of Table 2.2 shows that *Aspergillus niger* glucoamylase loses substantial activity at pH 1.6 to 1.8 after 21 hours relative to its activity at pH 5.6.

Also, the specification fails to provide guidance regarding the effects of pH treatment of any glucoamylase, and fails to provide guidance regarding the use of other glucoamylase polypeptides with an expectation that the activity of the glucoamylase will be inactivated by at least 50% or 90% at a pH of 1.0 to 1.7.

The quantity of experimentation needed to make or use the invention based on the content of the disclosure: While methods of making variants of a given polypeptide were known in the art at the time of the invention, e.g., mutagenesis, it was not routine in the art to screen for all genes as encompassed by the claims for those that encode a chymosin polypeptide having the desired activity under the recited conditions.

Thus, in view of the overly broad scope of the claims, the lack of guidance and working examples provided in the specification, the high level of unpredictability, and the significant amount of non-routine experimentation required, undue experimentation would be necessary for a skilled artisan to make and use the entire scope of the claimed invention. As such, appellant has not provided sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The scope of the claims must bear a

reasonable correlation with the scope of enablement (*In re Fisher*, 166 USPQ 19 24 (CCPA 1970)). Without sufficient guidance, determination of having the desired biological characteristics is unpredictable and the experimentation left to those skilled in the art is unnecessarily, and improperly, extensive and undue. See *In re Wands* 858 F.2d 731, 8 USPQ2nd 1400 (Fed. Cir, 1988).

Claim Rejections - 35 USC § 103

[12] The rejection of claims 60-62, 66-71, and 74-77 under 35 U.S.C. 103(a) as being unpatentable over the combination of Lawlis, Jr. et al. (US Patent 5,801,034; hereafter "Lawlis"; cited in the PTO-892 filed on 12/11/06) and Ward et al. (*Biotechnol* 8:435-440; cited in the IDS filed 16 April 2001; hereafter "Ward") is maintained for the reasons of record and the reasons set forth below. The rejection was fully explained in a prior Office action. See [11] beginning at p. 13 of the Office action mailed on 9/24/09.

RESPONSE TO ARGUMENT: Beginning at p. 13 of the instant remarks, applicant argues the rejection relies on a method of adding formic acid, however, formic acid is not recited in the claims.

Applicant's argument is not found persuasive. Claim 60 recites "[a] method…comprising the steps of" and because the transitional phrase "comprising" is inclusive, the claims do not exclude the addition of formic acid.

Applicant argues the combination of references does not teach all claim limitations because Lawlis does not teach lowering pH of a medium to between 1.0 and 1.7 with an inorganic acid.

Applicant's argument is not found persuasive. As noted in the prior Office action, Lawlis expressly teaches, adjusting the pH of a medium with a mineral acid, *i.e.,* inorganic acid, to about 1.75 *or less* (column 3, lines 51-60). However, even faced with this undisputed fact, applicant takes the position that it would not have been obvious to one of ordinary skill in the art to reduce the pH by 0.05 pH units to a pH of 1.7. According to MPEP 2144.05.II, "[g]enerally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical" and that "[a] particular parameter must first be recognized as a result-effective variable, i.e., a variable which achieves a recognized result, before the determination of the optimum or workable ranges of said variable might be characterized as routine experimentation". Here, there is no evidence that a pH of 1.7 (relative to a pH of 1.75) is critical to the claimed invention and the prior art makes clear that adjusting the pH to 1.75 *or less* is a result-effective variable, *i.e.,* effecting a cell kill. Thus, in accordance with MPEP 2144.05.II, it would have been obvious to one of ordinary skill in the art at the time of the invention to adjust the pH of a medium with a mineral acid to 1.7 using only routine experimentation.

Applicant argues there is no motivation to reduce pH below 1.75 because, according to the teachings of Lawlis, there is allegedly no significant benefit to lowering the pH more than 2 pH units below the pKa.

Applicant's argument is not found persuasive. As noted above, Lawlis expressly teaches adjusting the pH of a medium with a mineral acid, *i.e.,* inorganic acid, to about

1.75 *or less* (column 3, lines 51-60), which encompasses a pH of 1.7. As noted in the instant remarks at p. 14, top, Lawlis teaches, "By reducing the pH of the mixture…to a value equal to *or less* than two pH units below the pKa…the acid is 99% protonated…", which is an express teaching of using a pH more than two pH units below the pKa. As noted above, even faced with this undisputed fact, applicant takes the position that it would not have been obvious to one of ordinary skill in the art to reduce the pH by 0.05 pH units to a pH of 1.7. Even assuming *arguendo* one were to ignore Lawlis' express teachings of using a pH of 1.75 *or less* and reducing pH to a value equal to *or less* than two pH units below the pKa of formic acid, applicant acknowledges that "further reductions in pH would slightly increase the amount of protonated organic acid" (instant remarks at p. 14, middle) and thus one of ordinary skill in the art would have been motivated to reduce pH at least by 0.05 pH units with an expectation of achieving increased protonated acid and thus increased cell kill. Although applicant attempts to rely on Example IV of Lawlis in justifying this position, Example IV appears to be unrelated to a sulfuric acid/formic acid combination for cell kill and the conditions of Example IV are not relevant to a culture of *Aspergillus, i.e.,* the host cell of Example IV is a yeast, not a fungal host cell.

Applicant argues one would not have lowered the pH beyond what is necessary because of the potential inactivating effect of pH on enzyme activity.

Applicant's argument is not found persuasive. Initially, it is noted that the method as taught or suggested by the prior art is not directed to a generic "enzyme", but rather is specifically directed to *chymosin*. Here, there is no evidence of record that one of

ordinary skill in the art would have recognized chymosin as being susceptible to acid inactivation at a pH of 1.7 or that one of ordinary skill in the art would have expected such inactivation. To the contrary, chymosin was well-known in the prior art as an *acid* protease and was well-known to maintain catalytic activity, even at pH values well below 1.7. As noted in the Office action mailed on 12/19/02 at p. 7, bottom, the reference of Larsen (WO 95/29999; cited in the IDS filed on 4/16/01) teaches that a pH as low as 0.5 can be used to convert inactive chymosin to an active form.

Moreover, in the Decision on Appeal mailed on 5/19/09, the BPAI states, "A skilled worker would have reasonably expected that Lawlis' method would not adversely affect the activity of the chymosin produced by Ward's cells because Ward teaches that a pH of 2 actually increased the amount of active chymosin, and pH 1.75 is reasonably close to pH 2" (p. 18, bottom). Similarly, a skilled worker would have reasonably expected that Lawlis' method would not adversely affect the activity of the chymosin produced by Ward's cells because pH 1.7 is reasonably close to pH 2.

At least for the reasons of record and the reasons set forth above, it is the examiner's position that the claimed invention would have been obvious to one of ordinary skill in the art at the time of the invention.

[13] The rejection of claim 73 under 35 U.S.C. 103(a) as being unpatentable over the combination of Lawlis, Jr. et al. (US Patent 5,801,034; hereafter "Lawlis"; cited in the PTO-892 filed on 12/11/06) and Ward et al. (*Biotechnol* 8:435-440; cited in the IDS filed 16 April 2001; hereafter "Ward") is withdrawn in view of the instant amendment to claim

73 to replace the phrase "gene encoding chymosin is derived from *Camelus dromedarius*" with "gene encoding chymosin is from *Camelus dromedarius*", thus limiting the recited gene to encoding *Camelus dromedarius* chymosin.

[14] Claim 73 is rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Lawlis and Ward as applied to claims 60-62, 66-71, and 74-77 above and further in view of Wangoh et al. (*Milchwissenschaft* 48:322-325, 1993; hereafter "Wangoh"), and EMBL Accession Number AJ131677 (December, 2000; hereafter "EMBL AJ131677"). This rejection is necessitated by the instant claim amendment.

Claim 73 limits the recited gene of the method of claim 60 to encoding *Camelus dromedarius* chymosin.

The reference of Lawlis teaches, "[i]n the various processes of culturing or fermenting microorganisms, it is sometimes necessary during or at the conclusion of the fermentation process to be able to kill the active cells in the mixture so that the desired product can be recovered from the culture or fermentation mixture. This is particularly true when microorganisms containing recombinant DNA are grown as production hosts and it is desirable to prevent any viable recombinant organisms from being released into the environment" (column 1, lines 18-25). Lawlis teaches, "[i]n the development of this invention, it has been found that the change in pH alone of a fermentation mixture does not accomplish a complete or substantially complete cell kill. For example, in a culture of *Aspergillus niger* for the production of chymosin, reducing the pH to about 2 using sulfuric acid does not accomplish a complete or substantially complete cell kill"

(emphasis added; column 2, lines 58-64). To achieve a substantially complete cell kill, Lawlis teaches "selecting a compatible organic acid…adjusting the pH of the culture to a value equal to or less than about 2 pH units below the pK_a of a selected compatible organic acid and adding a sufficient amount of the selected compatible organic acid and/or salt (column 2, lines 29-39). Lawlis expressly teaches acetic acid, propionic acid, and formic acid as being used in the claimed method (see claim 2) and further teaches "if formic acid (pK_a =3.75) is to be used to accomplish the cell kill, the pH of the mixture will be adjusted with a mineral acid to about 1.75 or less, then formic acid is added to accomplish the cell kill" (column 3, lines 51-60). See also claims 2 and 3 of Lawlis, which specifically recites the use of formic acid as the organic acid and sulfuric acid as the mineral acid in the disclosed method. The working examples of Lawlis, although using acetic acid and not formic acid, teach that "substantially complete cell kill" can be achieved by overnight incubation (Example 1), a 60 hour incubation (Example 2), and a 4 hour incubation (Example III). Although Lawlis teaches the method be applied to a culture of *Aspergillus niger* for the production of chymosin (column 2, lines 58-64) and also specifically teaches adjusting the pH the of the mixture with a mineral acid, which as noted above is expressly taught as sulfuric acid in claim 3, to about 1.75 or less when formic acid is the selected organic acid (column 3, lines 51-60).

While Lawlis teaches and/or suggests applying the method to a culture of *A. niger* for the production of chymosin, Lawlis does not expressly teach or suggest applying the method to a medium comprising *Camelus dromedarius* chymosin and glucoamylase activities.

The reference of Ward teaches *Aspergillus niger* var. *awamori* comprises a gene encoding a glucoamylase polypeptide (p. 435, column 1, bottom; column 2, middle; and p. 437, column 2, top) and further teaches the use of an expression vector in which the cDNA encoding a bovine prochymosin B polypeptide was fused in frame immediately following the codon for the last amino acid of *Aspergillus niger* var. *awamori* glucoamylase gene and recombinant production of chymosin in *A. niger* var. *awamori* transformed with this vector "led to the secretion of considerably higher amounts of chymosin than obtained with previous chymosin vectors" (p. 435, left column, abstract). See also p. 437, right column, Table 2. According to Ward, the *A. niger* var. *awamori* medium comprising the secreted fusion exhibited chymosin activity and glucoamylase activity (p. 437, right column, Table 2).

While the combination of Lawlis and Ward teaches and/or suggests applying the method of Lawlis using the medium of Ward comprising *Aspergillus niger* glucoamylase and bovine chymosin, the combination does not expressly teach or suggest applying the method to a medium comprising *Camelus dromedarius* chymosin.

However, this is remedied by the references of Wangoh and EMBL AJ131677. Wangoh teaches that in the coagulation of camel milk, camel rennet should be used rather than bovine chymosin (p. 6, column 1). EMBL AJ131677 teaches an mRNA encoding *Camelus dromedarius* chymosin, which is annotated as "rennet coagulation of milk".

Therefore, at the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Lawlis, Ward, Wangoh and EMBL

AJ131677 to substitute bovine chymosin encoding nucleic acid with the nucleic acid of EMBL AJ131677 in the method of Ward and use a culture of the transformant in a method of Lawlis, namely, treating the culture with sulfuric acid to a pH of 1.7 and then adding formic acid to effect substantial cell kill. One would have been motivated to do this because Wangoh acknowledges the importance of using camel rennet in camel milk coagulation and EMBL AJ131677 acknowledges that *Camelus dromedarius* chymosin is an enzyme of rennet coagulation of milk. One would have had a reasonable expectation of success to substitute bovine chymosin encoding nucleic acid with the nucleic acid of EMBL AJ131677 in the method of Ward and use a culture of the transformant in a method of Lawlis because of the teachings of Lawlis, Ward, and EMBL AJ131677. Therefore, the method of claim 73 would have been obvious to one of ordinary skill in the art at the time of the invention.

The following comments are provided to clarify the instant rejection. Regarding the limitation, "subjecting said medium...for a period of time sufficient to inactivate at least 50% of said glucoamylase activity while maintaining at least 75% of said chymosin activity" in claim 9, according to MPEP 2111.01.I, "'[c]laims are not to be read in a vacuum, and limitations therein are to be interpreted in light of the specification in giving them their broadest reasonable interpretation'". Thus, although claim 73 does not specifically delineate the recited "period of time", the examiner has referred to the specification and claims to ascertain that which is intended as being encompassed by the "period of time". According to the specification at p. 7, lines 21-23, "[t]ypically…the required treatment period is within the range of 0.1 minutes to 48 hours", which is as

few as 6 seconds up to 48 hours. See also the limitations of claim 71, which limit the "period of time" in claim 60 to between 0.1 minutes to 48 hours. Accordingly, the examiner has interpreted the "period of time" to inactivate at least 50% or 90% of the glucoamylase activity, while maintaining at least 75% or 85% of the chymosin activity as being inclusive of 0.6 seconds up to 48 hours.

According to MPEP 2112, "[t]he express, implicit, and inherent disclosures of a prior art reference may be relied upon in the rejection of claims under 35 U.S.C. 102 or 103. 'The inherent teaching of a prior art reference, a question of fact, arises both in the context of anticipation and obviousness'". MPEP 2112.I states, "'[t]he discovery of a previously unappreciated property of a prior art composition, or of a scientific explanation for the prior art's functioning, does not render the old composition patentably new to the discoverer". MPEP 2112.IV states, "'To establish inherency, the extrinsic evidence must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill". While it is acknowledged that the combination of references fails to *expressly* teach inactivation of at least 50% or 90% of the glucoamylase activity, while maintaining at least 75% or 85% of the chymosin activity, this would be a necessary feature of practicing the method of Lawlis with a culture medium of the transformant of Ward producing a glucoamylase-chymosin fusion protein, particularly as Lawlis suggests treating a culture medium at a pH of 1.7 with overnight incubation or incubation for 4 hours, which pH and time are either specifically recited and/or disclosed in the specification to achieve the desired reduction in glucoamylase activity while

maintaining the desired level of chymosin activity. Thus, although the prior art does not *expressly* teach the noted limitation, since the pH and time of the prior art method are encompassed by the "period of time", practicing the prior art method would appear to necessarily result in the inactivation of at least 50% or 90% of the glucoamylase activity, while maintaining at least 75% or 85% of the chymosin activity as required by the claims.

[15] The rejection of claims 44-46, 50-51, 55, and 58-59 under 35 U.S.C. 103(a) as being unpatentable over the combination of Lawlis and Ward as applied to claims 60-62, 66-71, and 74-77 above and further in view of Chang ("Chemistry", McGraw Hill Inc., New York, 1991, p. 734), and Van Ooijen (US Patent 5,371,287; hereafter "Van Ooijen") is maintained for the reasons of record and the reasons set forth below. The rejection was fully explained in a prior Office action. See [12] beginning at p. 18 of the Office action mailed on 9/24/09.

RESPONSE TO ARGUMENT: Beginning at p. 17 of the instant remarks, applicant argues the pKa of lactic acid is 3.86, relying on the reference of "The Merck Index", 2006. According to applicant, two pH units below the pKa of lactic acid is 1.86, which is "outside of the claimed pH value".

Applicant's argument is not found persuasive. Initially, it is noted that applicant's evidence relied upon to support a pKa of lactic acid being 3.86 is published after the time of the invention, *i.e.,* 2006, and thus is not relevant to establishing the known pKa of lactic acid *at the time of the invention.* This is in contrast to the examiner's objective

evidence of Van Ooijen, which is prior art and supports a pKa of lactic acid being 3.08 (see Van Ooijen at column 1, line 50). See also Griffin et al. (*Am. J. Physiol. Heart. Circ. Physiol.* 279:H361-H367, 2000), which discloses lactic acid as having a pKa of 3.08 (p. H365, bottom), referencing the "CRC. Handbook of Chemistry and Physics" (77th ed.), edited by Lide DR. New York: CRC, 1997. Further, it is noted that applicant's asserted pKa value is actually disclosed in "The Merck Index", 2006 as "pK" rather than "pKa" and there is no evidence of record that these values are equal. Even assuming *arguendo* the prior art also discloses lactic acid as having a pKa of 3.86, one of ordinary skill in the art, recognizing a discrepancy in pKa values of 3.08 and 3.86, would have selected the *lower* of the two, *i.e.,* a pKa of 3.08 for lactic acid, in order to ensure substantial cell kill in accordance with the method of Lawlis.

At least for the reasons of record and the reasons set forth above, it is the examiner's position that the claimed invention would have been obvious to one of ordinary skill in the art at the time of the invention.

[16] The rejection of claim 57 under 35 U.S.C. 103(a) as being unpatentable over the combination of Lawlis and Ward as applied to claims 60-62, 66-71, and 74-77 above and further in view of Chang ("Chemistry", McGraw Hill Inc., New York, 1991, p. 734), and Van Ooijen et al. (US Patent 5,371,287; hereafter "Van Ooijen") is withdrawn in view of the instant amendment to claim 57 to replace the phrase "gene encoding chymosin is derived from *Camelus dromedarius*" with "gene encoding chymosin is from

Camelus dromedarius", thus limiting the recited gene to encoding *Camelus dromedarius* chymosin.

[17] Claim 57 is newly rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Lawlis, Ward, Wango, and EMBL AJ131677 as applied to claims 60-62, 66-71, and 73-77 above and further in view of Chang and Van Ooijen. This rejection is necessitated by the instant claim amendment.

The teachings of Lawlis, Ward, Wangoh and EMBL AJ131677 as applied to claims 60-62, 66-71, and 73-77 are set forth above. Lawlis further teaches "The 'organic acid' employed to effect a substantially complete kill can be any suitable and compatible acid having 1 to about 5 carbon atoms" (column 3, lines 30-32). Lawlis does not teach using lactic acid in the disclosed method.

The reference of Chang teaches lactic acid is a 3-carbon organic acid (p. 734, bottom).

The reference of Van Ooijen teaches the pKa of lactic acid is 3.08 (p. 4211, column 1).

At the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Lawlis, Ward, Wangoh, EMBL AJ131677, Chang, and Van Ooijen to use a culture of the transformant of Ward expressing *Camelus dromedarius* chymosin in a method of Lawlis, using lactic acid at a pH of 1.08. One would have been motivated to use lactic acid in the method of Lawlis because lactic acid satisfies the criteria of Lawlis, *i.e.,* it is an organic acid and has 3 carbons. One

would have been motivated to use a pH of 1.08 because Lawlis expressly teaches using a pH of a value equal to or lower than 2 pH units below the pKa of the organic acid (column 2, lines 32-34). One would have had a reasonable expectation of success to use lactic acid at a pH of 1.08 in the method of Lawlis because of the teachings of Lawlis, Ward, Wangoh, EMBL AJ131677, Chang, and Van Ooijen. Therefore, the method of claim 57 would have been obvious to one of ordinary skill in the art at the time of the invention.

[18] The rejection of claims 44-46, 50-55, and 58-59 under 35 U.S.C. 103(a) as being unpatentable over the combination of Lawlis and Ward is maintained for the reasons of record and the reasons set forth below. The rejection was fully explained in a prior Office action. See [13] beginning at p. 19 of the Office action mailed on 9/24/09. Claims 56 and 78 are included in the instant rejection in view of the amendment to the claim to limit the method of claim 44 to lowering the pH by addition of acetic acid and the addition of claim 78, which recites “acetic acid” as an organic acid. *Thus, claims 44-46, 50-56, 58-59, and 78 are rejected.*

RESPONSE TO ARGUMENT: Beginning at p. 17 of the instant remarks, applicant notes that the examiner’s response to argument at p. 20 of the Office action mailed on 9/24/09 states, "the combination of references as noted above teaches or suggests using lactic acid in the method of Lawlis", noting that this appears to be a reference to the rejection based on the combination of Lawlis, Ward, and Chang and requests clarification.

The examiner's acknowledges this statement is directed to the combination of Lawlis, Ward, Chang, and Van Ooijen.

Applicant argues Lawlis teaches away from using a pH lower than pKa - 2 pH units because further lowering of pH is ineffective to increase cell kill; Lawlis teaches enhancing cell kill only by increasing acid concentration without lowering pH and lowering pH risks losing enzymatic activity.

Applicant's argument is not found persuasive. As noted in the prior Office action, Lawlis expressly teaches a specific example of using acetic acid at a pH of 2.0 and further teaches using acetic acid at a pH of 2.75 *or less*. While Lawlis teaches the use of a pH of 1.75 for formic acid, one of ordinary skill in the art would have recognized the specifically exemplified pH of 1.75 as being a pH below 2.75 and thus applicable for acetic acid. According to MPEP 2144.05.II, "[g]enerally, differences in concentration or temperature will not support the patentability of subject matter encompassed by the prior art unless there is evidence indicating such concentration or temperature is critical" and that "[a] particular parameter must first be recognized as a result-effective variable, i.e., a variable which achieves a recognized result, before the determination of the optimum or workable ranges of said variable might be characterized as routine experimentation". Here, the prior art makes clear that adjusting the pH to 1.75 *or less* is a result-effective variable, *i.e.,* effecting a cell kill. Thus, in accordance with MPEP 2144.05.II, it would have been obvious to one of ordinary skill in the art at the time of the invention to adjust the pH of a medium with a mineral acid to 1.75 and add acetic acid using only routine experimentation.

Regarding applicant's argument that Lawlis teaches away from using a pH lower than pKa - 2 pH units because further lowering of pH is ineffective to increase cell kill, applicant's argument is not found persuasive at least because this statement appears to contradict the instant remarks at p. 14, middle, where it is stated, "…further reductions in pH would slightly increase the amount of protonated organic acid…"

Regarding applicant's argument that Lawlis teaches enhancing cell kill only by increasing acid concentration without lowering pH, applicant's argument is not found persuasive because Lawlis expressly exemplifies reducing pH to 1.75 with addition of organic acid to effect cell kill. As such, Lawlis clearly teaches enhancing cell kill by lowering pH.

Regarding applicant's argument that lowering pH risks losing enzymatic activity, applicant's argument is not found persuasive. As noted above, the method as taught and/or suggested by the prior art is not directed to a generic "enzyme", but rather is specifically directed to *chymosin*. Here, there is no evidence of record that one of ordinary skill in the art would have recognized chymosin as being susceptible to acid inactivation at a pH of 1.7 or that one of ordinary skill in the art would have expected such inactivation. To the contrary, chymosin was well-known in the prior art as an *acid* protease and was well-known to maintain catalytic activity, even at pH values well below 1.7. As noted in the Office action mailed on 12/19/02 at p. 7, bottom, the reference of Larsen (WO 95/29999; cited in the IDS filed on 4/16/01) teaches that a pH as low as 0.5 can be used to convert inactive chymosin to an active form.

Moreover, in the Decision on Appeal mailed on 5/19/09, the BPAI states, “A skilled worker would have reasonably expected that Lawlis' method would not adversely affect the activity of the chymosin produced by Ward's cells because Ward teaches that a pH of 2 actually increased the amount of active chymosin, and pH 1.75 is reasonably close to pH 2” (p. 18, bottom). Similarly, a skilled worker would have reasonably expected that Lawlis' method would not adversely affect the activity of the chymosin produced by Ward's cells because pH 1.7 is reasonably close to pH 2.

Beginning at p. 18 of the instant remarks, applicant argues the specification provides evidence of unexpected results where glucoamylase activity is unexpectedly reduced, while chymosin activity is maintained at pH 1.5 to 1.8. According to applicant, one would have rather expected lowering the pH to have inactivated all enzymatic activity.

Applicant’s argument is not found persuasive. Regarding the alleged unexpected results, this argument has been addressed in the Examiner’s Answer mailed on 1/25/08 at pp. 32-36, which is reiterated herein. Moreover, in the Decision on Appeal mailed on 5/19/09, the BPAI states, “Appellant has pointed to no evidence of record to support her argument that the effect of low pH on glucoamylase activity would not have been expected by those of skill in the art. An assertion of unexpected results must be supported by evidence, not just attorney argument or conclusory statements" (paragraph bridging pp. 19-20).

At least for the reasons of record and the reasons set forth above, it is the examiner's position that the claimed invention would have been obvious to one of ordinary skill in the art at the time of the invention.

[19] The rejection of claim 57 under 35 U.S.C. 103(a) as being unpatentable over the combination of Lawlis and Ward is withdrawn as noted above in view of the instant amendment to claim 57 to replace the phrase "gene encoding chymosin is derived from *Camelus dromedarius*" with "gene encoding chymosin is from *Camelus dromedarius*", thus limiting the recited gene to encoding *Camelus dromedarius* chymosin.

[20] Claim 57 is newly rejected under 35 U.S.C. 103(a) as being unpatentable over the combination of Lawlis and Ward as applied to claims 44-46, 50-55, and 58-59 above and further in view of Wangoh and EMBL AJ131677.

The teachings of Lawlis, Ward, Wangoh, and EMBL AJ131677 are set forth above. Lawlis further teaches that acetic acid is preferred due to its low cost and effectiveness (column 4, lines 49-53). Lawlis teaches that when acetic acid is used, the pH is adjusted to "about 2.75 *or below* by the addition of...sulfuric acid, then the acetic acid is added" (emphasis added). Lawlis teaches using acetic acid at a pH of 2.0 as an example of a pH *below* 2.75 (column 6, Example II). Lawlis does not teach using acetic acid at a pH of 1.75.

However, at the time of the invention, it would have been obvious to one of ordinary skill in the art to combine the teachings of Lawlis, Ward, Wangoh, and EMBL

AJ131677 to use a culture of the transformant of Ward expressing *Camelus dromedarius* chymosin and treating the culture with acetic acid at a pH of 1.75 to effect substantial cell kill. One would have been motivated to use the method of Ward for producing *Camelus dromedarius* chymosin because the method of Ward produces "considerably higher amounts of chymosin." One would have been motivated to use the method of Lawlis in order to effect substantial cell kill. One would have been motivated to use acetic acid because it is a "preferred" organic acid and one would have been motivated to use a pH of 1.75 because Lawlis expressly teaches using acetic acid at a pH of 2.75 *or less,* and while a pH of 1.75 is specifically exemplified with respect to formic acid, one of ordinary skill in the art would have recognized this pH as being a pH below 2.75 and thus applicable for use with acetic acid. One would have had a reasonable expectation of success to use a culture of the transformant of Ward expressing *Camelus dromedarius* chymosin and treating the culture with acetic acid at a pH of 1.75 to effect substantial cell kill because of the teachings of Lawlis, Ward, Wangoh, and EMBL AJ131677. Therefore, the method of claim 57 would have been obvious to one of ordinary skill in the art at the time of the invention.

Conclusion

[21] Status of the claims:

- Claims 44-78 are pending.
- Claims 44-78 are rejected.
- No claim is in condition for allowance.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to David J. Steadman whose telephone number is 571-272-0942. The examiner can normally be reached on Mon to Fri, 7:30 am to 4:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Manjunath Rao can be reached on 571-272-0939. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/David J. Steadman/
Primary Examiner, Art Unit 1656